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Localization of the Cytoplasmic Domain of the HIV-1 Accessory Protein Vpu Within the Profile Structure of a Phospholipid Monolayer

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Beamline(s): X22B

Introduction: The human HIV-1 genome encodes six accessory proteins. Vpu, one of those accessory proteins, is an 81 amino acid phosphoprotein with a N-terminal hydrophobic domain and a C-terminal hydrophilic domain. The biological function of Vpu concerns two different activities, the enhancement of the release of virus from the infected cell surface and the triggering of the degradation of the CD4 molecule in the endoplasmic reticulum. The enhancement of virus release is dependent on the transmembrane domain of Vpu which also exhibits nonspecific cation channel activity. In the case of degradation of CD4, the specific virus receptor on the cell surface, the hydrophilic domain of Vpu interacts with the cytoplasmic domain of CD4 thereby triggering the proteolysis of CD4. We have reported[1] a x-ray reflectivity study of Langmuir monolayers of a pure phospholipid, whose hydrocarbon chain length was selected to nearly match the length of Vpu's hydrophobic helix, and its mixtures with Vpu at the water/air interface. The reflectivity data as a function of decreasing lipid/protein mole ratio clearly indicates the mixing of the two components in the plane of the Langmuir monolayer in spite of the saturation of the long lipid hydrocarbon chains, consistent with the disordering of gel-phase domains as demonstrated directly by GIXD. The x-ray reflectivity data from these monolayers were analyzed by two totally independent methods, both employing the first Born approximation. The first was a slab model-refinement procedure which utilized a minimal number of slabs of constant density, where the parameters describing the slabs are varied to improve the agreement between the experimental data and that predicted by the model. Here, the Vpu contribution was treated as a systematic perturbation to the pure lipid monolayer profile structure as a function of increasing protein/lipid mole ratio. A second model-independent box-refinement procedure utilized only the finite extent of the monolayer structure normal to the air-water interface, which is known experimentally. The excellent agreement of the profiles obtained from the two methods firmly establishes the so-determined electron density profiles of the monolayers. Comparison of the electron density profiles as a function of increasing Vpu/phospholipid mole ratio at a constant, relatively high surface pressure of 45mN/m clearly indicated the contribution of the protein to the monolayer profile structure. Our interpretation of these profiles suggested that the transmembrane helix was localized within the hydrocarbon chains of the host phospholipid monolayer oriented approximately normal to the plane of the monolayer while the amphipathic helices of Vpu's cytoplasmic domain lie in the subphase parallel to the monolayer plane on the surface of the polar headgroups at this surface pressure.

Methods and Materials: Monolayers were spread in a custom-built Langmuir trough mounted on the sample stage of the Liquid Surface Spectrometer of X22B and x-ray specular reflectivity data were collected as described elsewhere[1].

Results and Conclusions: This interpretation has been confirmed by otherwise identical x-ray reflectivity studies at a monolayer surface pressure of 45mN/m of two sub-molecular fragments of Vpu[2], "Tmc" composed of residues 2-51 containing Vpu's transmembrane hydrophobic helix and only the first amphipathic helix of its cytoplasmic domain connected by a 3-residue loop, and "Tm" composed of residues 2-37 containing essentially only Vpu's transmembrane helix. These studies also explored a range of surface pressures thought to be physiologically relevant for the comparison of phospholipid monolayers and bilayers. We have now demonstrated that the localization of the amphipathic helices of Vpu's cytoplasmic domain depends strongly on the surface pressure over a relatively narrow range, being localized just below the phospholipid polar headgroup layer in the subphase at higher surface pressures (40-45mN/m) and within the polar headgroup layer at slightly lower surface pressures (30-35mN/m).

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